Fructose signalling unbalancing and effect on Arabidopsis growth and the photosynthetic pigments content

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SUMMARY

Sugar biosynthesis, tightly regulated in plants, depends on photosynthesis as the only biochemical process able to fix atmospheric CO_2 in photoautotrophic organisms. In *Arabidopsis thaliana*, sucrose, glucose, and fructose are the most abundant soluble sugars. Sugar pools are fluctuating during the day/night cycle responding to plant requirements, as the growth stage or the response to diverse environmental stresses. For that reason, plants have developed signalling mechanisms to orchestrate the replenishment (synthesis) and mobilization (hydrolysis) of the different sugar pools. The disruption of these signalling pathways can provoke either resistance or sensitivity to sugars. In this work, we have characterized several *A. thaliana* lines earlier selected as putative fructose insensitive. Our results have showed that, though deficient for fructose sensing/signalling, these plants exhibit normal physiological parameters of root growth and photosynthetic pigment accumulation. Nevertheless, after performing growth assays in a high glucose medium, some of these Arabidopsis lines have also revealed to be glucose insensitive.

INTRODUCTION (AND OBJECTIVES)

As photoautotrophic organisms, plants generate their own sugars through the photosynthesis, one of the most important processes for the life. During the day, the energy (ATP) and reducing power (NADPH) supplied from photosynthetic electron transport is used by the Calvin-Benson cycle to convert atmospheric CO_2 and water to carbohydrates and oxygen, using sunlight as an energy source. The energy-rich sugar molecules are used by plants for their development and growth. To maintain the balance of metabolite and energy levels, organisms have developed sophisticated sensing and signalling mechanisms that underlie the physiological responses to cell metabolite fluctuations.

Since few years, sugar sensing and signalling has become important in its effects on plant growth and development [1]. Complex metabolic and hormonal signals are cross talking to integrate and transmit vital information for plant adaptation to the changing environment. For example, high glucose concentrations inhibit growth and the establishment of photosynthesis in seedlings. On the contrary, low sugar concentrations promote growth. Still, a great deal remains to be learned about the precise molecular mechanisms involved. In a previous screening carried out in our laboratory, several T-DNA insertion Arabidopsis lines have been

selected as insensitive to high fructose concentrations (6%). In this work, we have characterized these Arabidopsis lines by analyzing some physiological parameters as the root-growth rate and the photosynthetic pigments content and tested whether, in addition to fructose, these lines are insensitive to other sugars as glucose.

MATERIALS AND METHODS

Plant material and growth conditions

Arabidopsis thaliana wild type (ecotype Columbia) and mutant plants were grown in soil in culture chambers under long-day conditions (16h light/ 8h darkness) at 22 ºC during the light and 20 ºC during darkness. *In vitro* plants were cultured on solid 0.5x Murashige and Skoog (MS) medium. The light intensity was 120 µmol m^{-2} s^{-1} .

Determination of photosynthetic pigments

After pigments extractions in methanol, the content of chlorophyll *a* (chla) and *b* (chlb), and carotenoids was spectrophotomerically quantified according to the method of Lichtenthaler and Wellburn (1983) [2].

RESULTS

Gene mutations leading to fructose insensitivity

Eight Arabidopsis mutant lines (homozygous T-DNA insertion lines from SALK laboratory) were selected as high-fructose concentration insensitive, according to a previous selection (unpublished data). An *in silico* search was developed to find out which genes were responsible for this resistance. The gene list is showed in Table 1. Genes coding for putative nuclear proteins (At3g10530, At5g48090, and At1g56240), putative oxidoreductases (At2g38080 and At1g15140), a putative heat shock protein (At3g62600), a putative stress protein (At2g47710), and a protein of unknown function seem to be involved in the resistance behavior. In line with our results, a nuclear protein (a transcription factor) has already been described as one important player in the fructose signalling pathway in *A. thaliana* [3]. As can be observed in figure 1, these Arabidopsis mutants show a wild-type phenotype at the seedling stage.

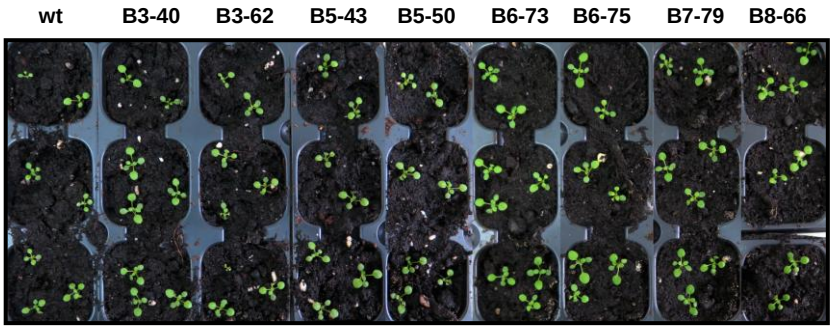

Figure 1. Phenotype of wild-type plants and *A. thaliana* mutant lines 14 days after germination (dag).

Determination of chlorophyll and carotenoid content

Photosynthesis homeostasis depends on a proper and balanced chlorophylls (chl) and carotenoids content. Moreover, deviations in the normal chl *a*/*b* ratio could suggest

photosynthesis alterations [4]. In order to know whether these mutant lines have photosynthesis impairments, photosynthesis pigments were isolated from plants showed in figure 1 and spectrophotometrically quantified. In figure 2A, it can be observed a similar pigment content in all lines, including wild-type. However, chlorophylls ratio (figure 2B) of some mutant lines (B3 40, B5 50, and B6 73) are slightly different from wild-type, pointing out to possible alterations in photosynthesis that should be further investigated.

Analysis of the *in vitro* root growth

In order to know whether these Arabidopsis lines were developmentally affected, seedling root growth was tested in control (sugar-free) or in sucrose-containing (1% sucrose) solid media. Figure 3 shows that there were no significant differences among the lines analyzed neither in the free-sugar medium nor the sucrose-containing medium. As it could be expected, the rate growth in the presence of sucrose was higher than in the absence of this sugar (approximately 2 fold).

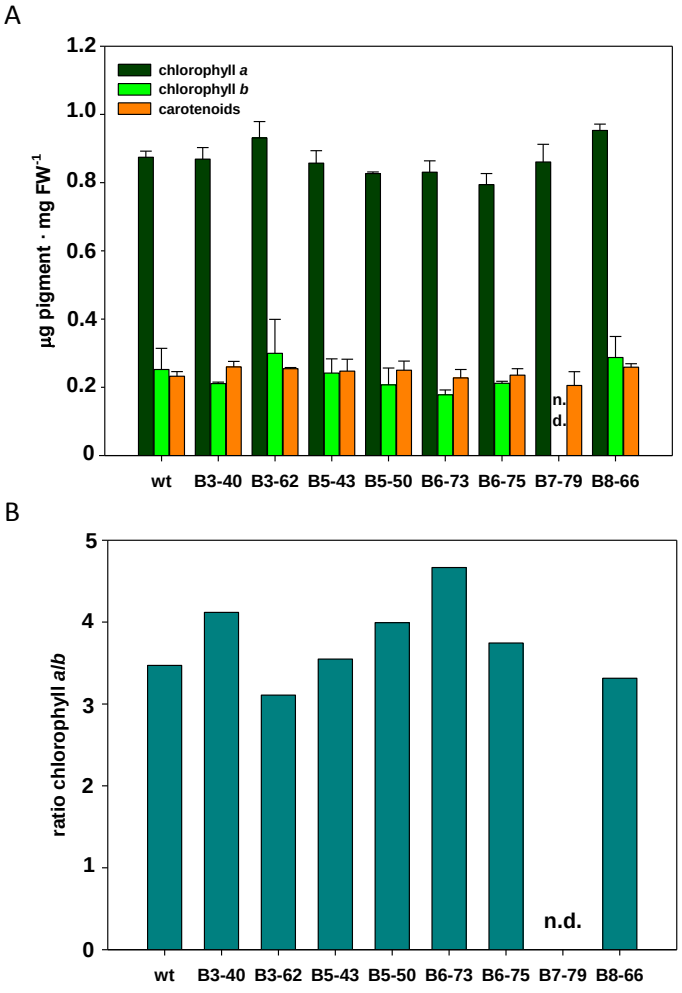

Figure 2. Photosynthetic pigments in *A. thaliana* lines grown in soil. A, Chlorophyll *a* and *b* and carotenoids content in 14-dag wild-type and mutant plants. B, Calculation of the chlorophyll *a*/*b* ratio for the different lines. At least five plants were analyzed for each measurement. n.d., not determined.

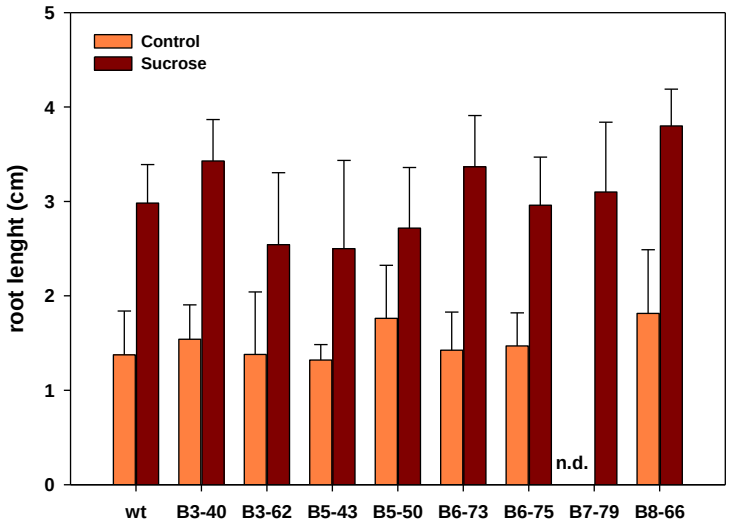

Figure 3. Root length of the Arabidopsis lines (wild-type and mutants) of 7-dag plants grown on 0.5x MS medium with or without sucrose (1%). Seedlings were grown vertically on the surface of hard agar plates. At least five plants were analyzed for each measurement.

Table 1. Identification of the loci containing the T-DNA insertions and their putative functions. Data were obtained from TAIR database (www.arabidopsis.org).

Assigned ID	AGI affected	Putative function
B3-40	At2g38080	Hydroquinone:oxygen oxidoreductase activity.
B3-62	At3g10530	Nuclear protein.
B5-43	At3g62600	Unfolded protein binding, heat shock protein binding.
B5-50	At5g48090	Nuclear protein.
B6-73	At1g56240	Nuclear protein.
B6-75	At1g15140	Oxidoreductase activity.
B7-79	At2g47710	Putative stress protein.
B8-66	At5g25750	Unknown function.

Determining sugar specificity of the putative signalling/sensing pathways altered in the different Arabidopsis lines

Mutations leading to gaining insensitivity to a high fructose concentration in the growth medium could be specific of this sugar or might be rather an unspecific resistance to several sugars. With the purpose of differentiating both possibilities, Arabidopsis mutant lines were grown in solid medium containing a high glucose concentration (6%) and compared with plants grown in the presence of the same fructose concentration. In figure 4 can be observed that lines B3-40, B5-43, and B6-73 are resistant to both sugars. Surprisingly, and on the contrary to our previous results, the lines B7-79 and B8-86 were fructose sensitive. Only Arabidopsis lines B3-66, B5-50, and B6-75 showed specific fructose insensitivity, corresponding to mutations in genes At3g10530 and At5g48090 (two putative nuclear proteins) and At1g15140 (a putative oxidoreductase), respectively. As expected, wild-type plants had highly impaired germination on the fructose medium while on the glucose medium the germinated seedlings showed white cotyledons (no photosynthetic activity).

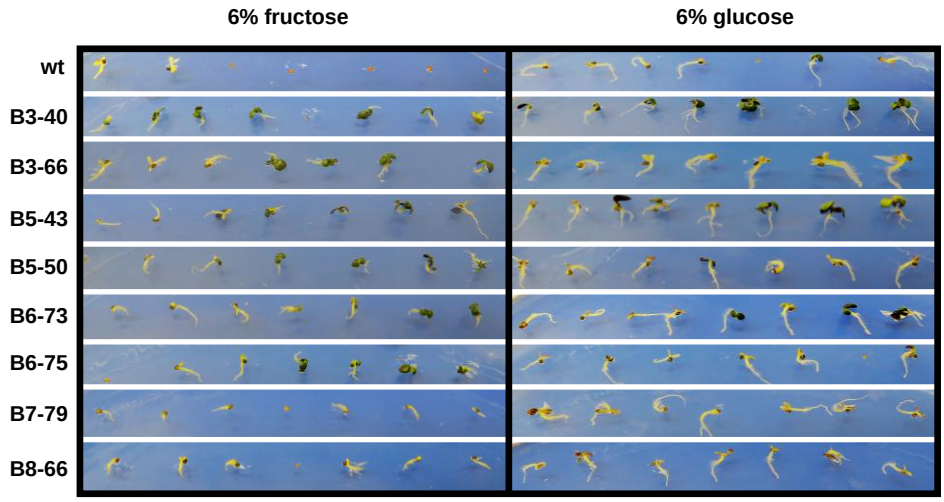

Figure 4. Fructose and glucose signalling. Arabidopsis lines were grown for 12 days on 0.5x MS medium containing 6% fructose or glucose.

CONCLUSIONS

[1] Despite the link between photosynthesis and sugar biosynthesis, our results show that disturbing sugar signaling does not necessarily affect Arabidopsis photosynthetic pigment content or the plant growth.

[2] In addition to being fructose insensitive, a 37.5% of the mutant lines also showed glucose insensitivity. The presence of 25% false positives in a second screening round suggests that environment factors could greatly affect the screening results.

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MY OWN IDEAS

Rafael Barnes Ontiveros, IES Francisco Ayala, Granada
En este proyecto hemos estado estudiando el efecto de los azúcares sobre el crecimiento vegetal y contenido de las plantas en Arabidopsis thaliana. Durante nuestro proyecto hemos tenido que llevar a cabo diferentes tareas tales como: siembra de semillas en medios de cultivo, medición de raíces y recuento de hojas, determinación del contenido de las clorofilas.
Para poder conseguir los resultados que nos hacían falta para completar el proyecto, hemos tenido que aprender a usar el método científico, con todo lo que conlleva, y en especial, el uso de herramientas y técnicas de laboratorio.
Para mí poder participar en este proyecto de investigación ha supuesto una fantástica experiencia que me ha permitido conocer cómo es el mundo de la ciencia, de lo que estoy muy satisfecho porque estoy seguro de que en un futuro me ayudará a no partir de cero y elegir mejor.

Marina Fiñaga Cortés, IES Federico García Lorca, Churriana de la Vega
In this project we worked with the model plant *Arabidopsis thaliana*, both with wild type as with some derived lines.
During the first session we sow seeds on agar culture media containing different sugars: glucose, fructose and sucrose. We let them grow for about 10 days in a growth chamber with a photoperiod of 16 hours light and 8 dark and 22ºC of temperature. Then, we carried out a set of experiments in order to analyse the effect of different sugars on the plant physiology. These experiments consisted in counting the leaf number and quantifying the rosette biomass, determining the root-growth rate and the chlorophyll content. Finally, we statistically analysed the results and obtained conclusions.

En este proyecto hemos trabajado con la planta modelo Arabidopsis thaliana, tanto con la forma silvestre como con diferentes líneas derivadas de ésta.
En la primera sesión sembramos semillas en cajas de cultivo in vitro con agar que contenían diferentes azúcares: glucosa, fructosa y sacarosa. Cultivamos las plantas durante 10 días en una cámara de cultivo con un fotoperiodo de 16 horas de luz y 8 horas de oscuridad a 22ºC. A continuación analizamos el efecto de los azúcares sobre la fisiología de la planta. Estos experimentos consistieron en contar el número de hojas y cuantificar la biomasa de la roseta, determinar la tasa de crecimiento de las raíces y el contenido de clorofila. Finalmente se analizaron estadísticamente los resultados y obtuvimos una serie de conclusiones.

María Elena Noguera Vilches, CDP Sagrado Corazón, Granada
This experience has helped me to get more into the field of plant biology. This project has been amazing because I never imagined that we would be able to observe consequences over the plant physiology coming from small modifications in the DNA. This project showed how is the work in a laboratory and also how important is to follow the methodology, the order and, especially, the English language. I am very happy to have participated in this experience.

Esta experiencia me ha ayudado a profundizar en el campo de la biología de las plantas. Este proyecto ha sido sorprendente ya que nunca imaginé que seríamos capaces de observar consecuencias derivadas de pequeñas modificaciones en el ADN. Este proyecto me ha enseñado cómo se trabaja en un laboratorio de investigación y la importancia que tiene la metodología, el orden y, especialmente, el inglés. Estoy muy contenta de haber participado en esta experiencia.

Claudia Rodríguez Ibáñez, CDP Sagrado Corazón, Granada

In this project we have studied the *Arabidopsis thaliana p*lant, both wild type as modified lines. Specifically, we have studied how different kind of sugars like glucose, sucrose or fructose affects the plant growth. To do that, we let grow the plants for 10 days in a culture house with 16 hours light and 8 dark with a temperature of 22 °C. After that, we compared the different plant lines and we did a detailed study generating graphs and obtaining conclusions. I chose this project because it provoked me a great interest and curiosity. The variety of knowledge acquired during this work has helped me to understand and increase my expertise on scholar subjects that were either unknown or partially known for me. Particularly, I have learned how is the work in a research laboratory.

En este proyecto hemos estudiado la planta Arabidopsis thaliana, tanto la variedad silvestre como otras variedades derivadas de la misma. Concretamente hemos estudiado cómo diferentes azúcares como la glucosa, la sacarosa o la fructosa puede afectar al crecimiento de las plantas. Para llevar a cabo este estudio se cultivaron plantas de Arabidopsis con un ciclo de luz de 16 h y de oscuridad de 8 h a 22ºC. Después se compararon las diferencias entre líneas y realizamos un detallado estudio generando distintas gráficas y obteniendo diversas conclusiones. Escogí este proyecto porque suscitó en mi mucho interés y curiosidad. La variedad de conocimientos adquiridos durante este trabajo me ha ayudado a entender e incrementar mi experiencia en temas académicos que desconocía total o parcialmente. Sobre todo he aprendido como se desarrolla el trabajo en un laboratorio de investigación.